Development of Self-incompatible Lines with Improved Seed Quality in Winter Oilseed Rape (*Brassica napus* L.) for Hybrid Breeding

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Abstract: Doubled haploid (DH) self-incompatible (SI) regenerants with improved seed quality were derived after two improvement crossing cycles with different "00" quality donors. The original genetic resource of recessive sporophytic SI was characterised by a high glucosinolate (GSL) and erucic acid content. SI regenerants of satisfactory seed quality parameters were obtained from the second cycle of crossing. Total number of derived SI plants was 87, i.e. 38.2% out of 228 obtained fertile doubled haploid regenerants developed in 2004. Nine plants out of the analysed 45 SI DH regenerants had the erucic acid content lower than 2%. Five out of these nine plants, which were analysed by HPLC method, had the glucosinolate content lower than the limit 18 μmol/g at 9% seed moisture. The method based on the spraying of inflorescences with 5% NaCl to enable reproduction of SI lines was verified.

Keywords: oilseed rape (*Brassica napus* L. var. *napus*); hybrid breeding; self-incompatibility; seed quality; glucosinolates; erucic acid

Several authors (Sernyk 1983; Thompson 1983; Grant & Beversdorf 1985; Lefort-buson *et al*. 1987; Paul *et al*. 1987; Paulman & Frauen 1991; Mohring *et al*. 1998) demonstrated hybrid vigour and the possibility of its utilisation in rapeseed (*Brassica napus* L. ssp. *napus* f. *biennis*). Cytoplasmic male sterility (CMS), genic male sterility (GMS) or self-incompatibility (SI) has been utilised in practical breeding of hybrid cultivars. In the Czech Republic, hybrids on the basis of male sterility systems MSL (Männlicher Sterilität Lembke) and Ogu-INRA are registered at present. Self-incompatibility is defined as the inability of a fertile hermaphrodite seed plant to produce zygotes after self-pollination. The sporophytic SI system in the *Brassica* family is controlled by multiallelic *S* locus, which determines specificity of interaction between pollen and cells of stigma (Nettancourt 1977). According to classical genetic analysis based on phenotype effect, *S* alleles in *Brassicaceae* are divided into the allele group with a high level of dominance and into the group of recessive alleles (Nasrallah *et al*. 1991). Gemmell *et al*. (1989) found nine different *S* alleles controlling sporophytic SI in *Brassica napus*. About twenty genes exactly cosegregated with SI phenotype were identified in *S* locus (Boyes *et al*. 1997; Casselman *et al*. 2000). Schweiger and Rudloff (1981) observed natural occurrence of SI in winter rapeseed and they found about 1% SI plants in populations of

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oilseed rape. However, the low stability of SI and undesirable oil and meal quality in their progeny were found by the above-mentioned authors. Havel (1994, 1996) detected the frequency of SI plants in several oilseed rape cultivars to be about 1%, but the 90% of the progeny from the SI plants was self-compatible (SC). Even though many of self-incompatible plants showed unstable SI behaviour, six stable SI lines were found. Four of the detected lines had different *S* alleles, in the other two lines identification of *S* alleles was not carried out. Esch and Wricke (1995) reported the frequency of SI plants in oilseed rape populations about 0.08%.

The original S homozygous lines developed from double low quality cultivars (Havel 1994) were distinguished for a high glucosinolate (GSL) and erucic acid content. During successive generations of selection for quality improvement in hybrids of those lines with 00 quality donors SI gradually vanished. Therefore, the use of the method of microspore cultures for obtaining improved SI lines was considered. The occurrence of SI plants out of the whole amount of DH regenerants derived from F1 hybrids of SI lines with 00 quality donors was 10–27% although the expected ratio of SI to SC plants was 1:1 (Kučera *et al*. 1997). Ziemborska and Harney (1986) also detected the number of self-compatible plants to amount to 71–93% in DH regenerants from anther cultures of *Brassica napus* var. *napobrassica*.

The routine methods such as seed-set test and fluorescence microscopic test, based on observing the pollen tube growth in UV light, have been used for detection of SI plants (Nettancourt 1977). Havel (1996) searched for SI lines by means of fluorescence and seed-set methods. The seed-set test proved better detection of SI than the fluorescence method that was more time consuming and its results were often distorted. The occurrence of SI plants in the population of low erucic acid and GLS content cultivars ranged between 0.3 and 3.9% in dependence on the year (Havel 1994).

For the maintenance and reproduction of SI lines on a large scale, it is necessary to overcome the SI reaction in open flowers after self-pollination by means of a chemical treatment. An increased concentration of CO_2 was recommended to obtain a smaller amount of seeds (Nakanishi & Hinata 1973). Fu *et al*. (1992) and Mohring *et al*. (1998) demonstrated the overcoming of self-incompatibility in oilseed rape by spraying inflorescences with 2–10% NaCl solution. In the field conditions, 5% NaCl solution applied every 3–5 days was enough for the practical reproduction of SI lines.

The aim of this study was to produce lines with a high self-incompatibility degree and desirable double zero quality.

MATERIAL AND METHODS

Quality improvement of SI lines. Doubled haploid regenerants derived from the F1 hybrids of SI line Tandem 6/85 and "00" quality donor OP-2051 after the first cycle of crossing were developed in 2000 at the Research Institute of Crop Production (RICP), Prague-Ruzyně. The initial line Tandem 6/85 with high GSL and erucic acid content in seed was obtained at the workplace OSEVA PRO Ltd., Research Institute of Oilseed Crops (RIOC) at Opava. Assessment by minimising methods of antinutritional substances in the seed of DH regenerants was carried out after verification of SI reaction by the seed test at RIOC.

For the second cycle of 00 quality improvement, five crosses between the best two SI DH lines OP-23 AI/3 and OP-23 AI/6 and 00 quality donors were performed in 2002. The rapeseed lines originated from RIOC (OP-BN-03, OP-571/00) and registered varieties (Rasmus and Lisek) were used as the male parents. From these crosses, 87 DH SI regenerants were derived at RICP in 2004. According to the amount of harvested seeds, 45 SI plants were selected for the assessment of erucic acid content. Out of the nine regenerants with erucic acid content lower than 2% five plants providing a sufficient amount of seeds were analysed for glucosinolate content by HPLC method.

Production of doubled-haploid regenerants from microspore cultures. Doubled haploid regenerants were produced by a microspore culture method (Vyvadilová & Zelenková 1992). The method was optimised for routine usage in winter oilseed rape and cruciferous vegetables breeding programmes (Klíma *et al*. 2004).

Determination of glucosinolate content. The assessment of glucosinolate content in seeds of oilseed rape was carried out according to the method of glucosinolate content assessment in rapeseed by high-pressure liquid chromatography (HPLC). The method that is deduced from the International Standard ISO 9167-1:1992 (E) (1992) was partially modified for the purposes of single seed analysis. The analyses were carried out on

liquid chromatograph Spectra-Physics, type SP 8100 XR with the detector SP 8440 XR UV/VIS and the integrator SP 4200.

Determination of fatty acid content. Individual fatty acid (FA) assessment in seeds of oilseed rape was carried out according to the method of single fatty acid assessment in rapeseed oil using gas chromatography (GC) modified for the analysis of single seed sample (Kolovrat 1985). Methyl esters of fatty acids were analysed on gas chromatograph CHROM 5.

Verification of SI line reproduction by means of overcoming SI. The possibility of seed production in SI lines by spraying flowers with 5% NaCl solution was verified in 2002. The SI lines OP-23 AI/3 and OP-23 AI/6 were chosen for the trial. All the plants were grown under the conditions of pot trials and technical isolation. The lines were isolated under a technical isolating cage from unwoven fabric 2 × 3 × 1.8 m in size. The spraying was carried out 3 times per week during the flowering period. The number (percentage) of produced seeds of SI lines after NaCl treatment was determined by the method of mean weight of SI plant seeds compared with the produced seeds of plants of OP-23 AI/3 and OP-23 AI/6 crossed with fertile line OP-2051. The mean weight of produced seeds of F1 plants was taken as 100%.

RESULTS AND DISCUSSION

SI line quality improvement by the method of doubled haploid microspore regenerant production

A partial decrease in glucosinolate and erucic acid content was achieved in 10 DH regenerants derived in 2000 from the first cycle of crossing for seed improvement of original SI lines. Only two lines, OP-23 AI/3 and OP-23 AI/6, had a significantly lower erucic acid content than the initial SI material Tandem 6/85. The average GSL content in this set of regenerants was 84.68 μmol/g of dry matter of seeds and the average erucic acid content was 22.84% (Table 1). Seed quality analyses did not prove a possibility of achieving 00 quality hybrids using partially improved SI lines as parents. Therefore, the production of DH microspore regenerants from the second cycle of hybrids of SI and quality donors was started from 2003 (Table 2). In total, 468 regenerants were derived in 2004, out of them 228 (48.7%) with fertile flowers. The

Table 1. Contents of antinutritional substances in seeds of parental components and SI DH regenerants derived from F1 hybrids in 2000 (the first improvement crossing cycle)

Genotypes/plant designation	Glucosinolate content (μmol/g dry matter of seed) HPLC	Content of erucic acid (%) GC
Tandem 6/85 (AI)	116.31	45.78
OP-2051 (donor of 00 seed quality)	7.96	0.53
OP-23 AI/2	102.06	24.88
OP-23 AI/3*	83.84	3.51
OP-23 AI/5	81.47	33.51
OP-23 AI/6*	80.99	3.20
OP-23 AI/8	95.28	23.88
OP-23 AI/10	71.00	21.76
OP-23 AI/11	99.21	33.98
OP-23 AI/13	78.83	28.40
OP-23 AI/18	75.05	24.11
OP-23 AI/22	79.19	31.17
Mean of OP-23 AI	84.692	22.84

*Selected SI plants used for experimental hybrids and the second cycle of improvement crossing with 00 donor of seed quality

Table 2. The origin of SI lines from the second improvement crossing cycle, harvested in 2004

Genotype	Origin (cross combination)
OP-11	OP-23 AI/6 × Rasmus
OP-12	OP-23 AI/6 × OP-BN-03
OP-13	OP-23 AI/6 × Lisek
OP-14	OP-23 AI/3 × Rasmus
OP-15	OP-23 AI/3 × OP-571/00

rest comprised sterile haploids and aneuploids. Out of the total number of fertile DH regenerants, 87 plants (38.2%) showed to be SI according to the seed-set test. The occurrence of SI plants out of the total number of fertile DH regenerants ranged from 32.4 to 45.5% depending on the origin of cross combination (Table 3). The earlier results (Havel 1994) showed that in F_2 generation derived from SI hybrids the segregation ratio of SI to SC plants was 1:15. This indicates recessive self-incompatibility controlled by two allelic pairs. In the population of 228 fertile DH regenerants, obtained in 2004, the segregation ratio of SI:SC plants was 1:1.62, which did not correspond with either monogenic or digenic base of observed SI. The expected ratio of SI to SC regenerants was approximately 1:1. However, a considerable shift to self-compatibility was observed, probably due to gametic selection against SI genotypes in the microspore culture (Kučera *et al*. 2002). In addition to this, modifier genes (Hinata *et al*. 1983) could influence the expression of self-incompatibility. Therefore, a suitable genetic background should be found for each *S* allele in which SI is strongly and stably expressed (Mohring *et al*. 1998).

In the analysed set of 45 SI DH regenerants, derived in 2004, nine regenerants showed the erucic acid content lower than 2% and the remaining

Table 3. Proportions of SI plants out of the total amount of microspore regenerants obtained in 2004 (according to the seed-set test)

Genotype	Total amount of derived regenerants	Fertile DH plants		SI out of the total of fertile plants	
		total amount	%	number	%
OP-11	95	56	58.9	21	37.5
OP-12	129	66	51.2	30	45.5
OP-13	54	27	50.0	9	33.3
OP-14	89	37	41.6	12	32.4
OP-15	101	42	41.6	15	35.7
Total	468	228	48.7	87	38.2

Table 4. Number of analysed SI DH regenerants with confirmed SI reaction (according to the seed-set test) including results of quality analyses

Genotype	Number of plants analysed for GSL/erucic acid	Erucic acid content (%) GC	Glucosinolate content (μmol/g of seed at 9% moisture) HPLC	Number of selected SI DH regenerants of 00 quality
OP-11	0/6	3.47–29.93	*	0
OP-12	5/22	0.13–28.34	3.59–18.76	5
OP-13	0/3	1.01–35.56	*	0
OP-14	0/9	1.24–29.00	*	0
OP-15	0/5	3.43–30.42	*	0
N total	5/45	–	–	5
Mean	–	13.78		–

*Samples without GSL content assessment due to the lack of seed material

Table 5. Results of seed quality analyses of selected SI DH regenerants with 00 seed quality harvested in 2004

Genotype	Plant number	Erucic acid content (%) GC	GSL content (μmol/g of seed at 9% moisture) HPLC
	2	0.13	14.84
	5	0.37	3.59
	42	1.06	*
OP-12	49	1.15	*
	53	1.12	13.99
	58	1.38	13.23
	64	1.39	9.42
OP-13	7	1.01	*
OP-14	42	1.24	*

*Samples without GSL content assessment due to the lack of seed material

36 regenerants over 2%. The average erucic acid content in the set of DH regenerants was 13.78%. Five out of the selected nine plants had GSL content lower than the limit 18 μmol/g at 9% seed moisture (Table 4). Table 5 shows seed quality parameters of selected SI DH regenerants with satisfactory 00 seed quality. The results of analyses indicate that in the case of SI DH regenerants from the second cycle of crossing with successive DH deriving the critical factor of seed quality was erucic acid content contrary to GSL content.

As reported by Havel (1994, 1996) and Kučera *et al*. (1997), it was difficult to achieve a desired combination of SI and double zero quality in oilseed rape lines by means of conventional breeding procedures. Therefore, the technique of doubled haploids that makes it possible to create lines with traits that are difficult to combine was used. The method proved to be applicable for the seed quality improvement of DH lines together with maintaining of SI reaction.

Verification of the method of overcoming SI

For the maintenance and reproduction of SI lines to create experimental or commercial hybrids, the overcoming of SI reaction is a basic necessary precondition. The obtained results of spraying inflorescences of rape plants with 5% NaCl solution for seed production of SI lines are in accordance with the statements of Fu *et al*. (1992) and Mohring *et al*. (1998). The seed production

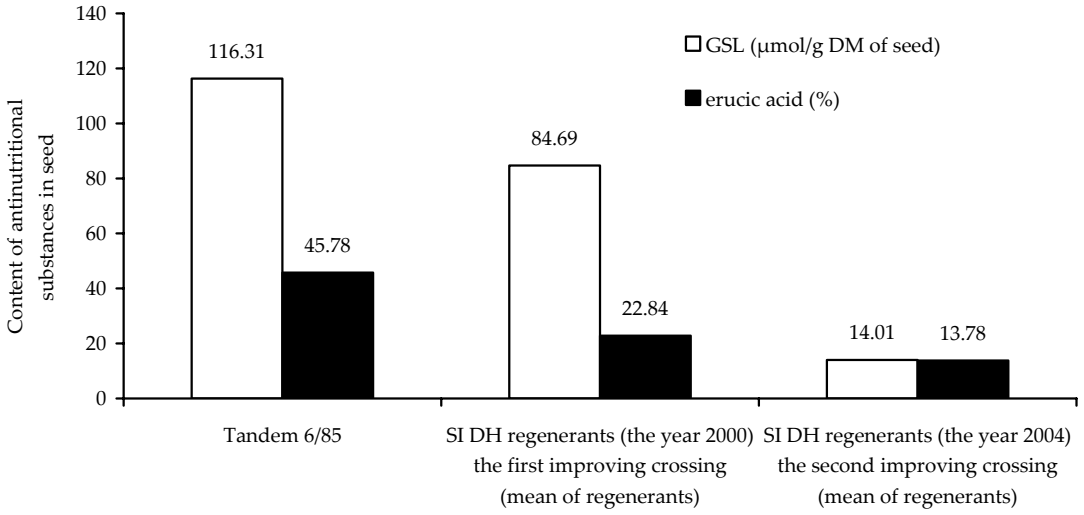

Figure 1. Seed quality of initial SI line Tandem 6/85 and two generations of SI DH regenerants derived from F1 hybrids with donors of 00 quality

in the line OP-23 AI/3 was 14.3% and in OP-23 AI/6 16.4% in comparison with the mean weight of seeds obtained from SI lines crossed with fertile line OP-2051 (100% was 26.87 g/plant). The average seed set of both lines was 4.12 g/plant (15.35%).

CONCLUSION

The production of doubled haploids by the technique of microspore cultures applied to crosses between SI lines and "00" seed quality donors could be a suitable method for obtaining improved SI lines of convenient quality. Two populations of SI DH regenerants were derived. In each of the following cycles of DH regenerant production, GSL and erucic acid content in seed decreased (Figure 1). SI plants with antinutritional substances under the limit value were obtained after the second cycle of DH regenerant development. According to the results of analyses, five plants complied with the quality requirements for GSL and erucic acid content. Obtained SI lines with improved quality have been used for self-incompatibility research purposes and for creating new experimental SI hybrids.

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Abstrakt

Koprna R., Kučera V., Kolovrat O., Vyvadilová M., Klíma M. (2005): **Vytváření výchozích autoinkompatibilních linií ozimé řepky se zlepšenou kvalitou semene pro šlechtění hybridů**. Czech J. Genet. Plant Breed., **41**: 105–111.

Z výchozích genetických zdrojů recesivní sporofytické autoinkompatibility (AI) u řepky ozimé byly po dvou cyklech zlepšovacího křížení s různými donory 00 kvality odvozeny dihaploidní (DH) autoinkompatibilní regeneranty z mikrosporových kultur. Výchozí genetický zdroj AI se vyznačoval vysokým obsahem glukosinolátů (GSL) a kyseliny erukové v semeni. Po druhém cyklu křížení byly získány AI regeneranty s vyhovující 00 kvalitou. Celkový počet odvozených AI regenerantů byl 87 (38,2 %) z 228 fertilních dihaploidních regenerantů. Z analyzovaného souboru 45 AI DH regenerantů, vytvořených v roce 2004, mělo 9 rostlin obsah kyseliny erukové do 2 %. Analýza GSL metodou HPLC prokázala u pěti z těchto rostlin obsah GSL nižší než limit 18 μmol/g semene při 9% vlhkosti. Byla též ověřena praktická použitelnost metody produkce semen AI linií postřikem 5% roztoku NaCl v podmínkách technické izolace.

Klíčová slova: řepka ozimá (*Brassica napus* L. var. *napus*); hybridní šlechtění; autoinkompatibilita; kvalita semen; glukosinoláty; kyselina eruková

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